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Modification of the American Public Health Association Procedure for Counting Yeast and Mold in Cottage Cheese

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The American Public Health Association method for counting low numbers of yeast and mold in cottage cheese was unsatisfactory due to altered *p*H of the culture medium. A modification of this method is presented.

Problems in enumerating yeast and mold were encountered during military subsistence testing of cottage cheese. These problems have resulted from the changes made in the American Public Health Association (APHA) 12th edition of *Standard Methods for the Examination of Dairy Products* (2). Military Subsistence Testing Laboratories must comply with these changes since the APHA method is cited in Military Specification MIL-C-43274A (3).

The APHA method (2) for counting yeast and mold in cottage cheese calls for diluting the cheese 1:10 with 2% sodium citrate which has been preheated to 40 C. Where the count is expected to be low, a 10-ml amount is distributed evenly among three petri plates and poured with acidified (*p*H 3.5) Potato Dextrose Agar (PDA, Difco). This procedure raises the *p*H of PDA sufficiently to permit bacterial growth, so that it is difficult to distinguish yeast from bacterial colonies. Previous to the 12th edition, the APHA method (1) prescribed a 1:2 dilution of cottage cheese in 2% sodium citrate; 1 ml was then distributed into each of two petri plates. Although the PDA remained inhibitory to bacterial growth, the heavy suspension clouded the medium and made the petri plates difficult to read. The present investigation was undertaken to improve the APHA method for counting yeasts and molds in cottage cheese.

Table 1 shows the effect of plating 1 ml and 3.3 ml of a 1:10 dilution of cottage cheese in 2% sodium citrate on the *p*H and bacterial inhibition of acidified PDA. Bacterial growth occurred only when 3.3 ml of the cheese was cultured. This was due to the increase in *p*H of PDA from 3.5 to 5.6 when 10 ml of the medium was poured as recommended by APHA (2). Even when 20 ml of PDA was poured, the *p*H of the medium went as high as 4.9, which also permitted bacterial growth. The 1:10 cheese-citrate dilution had a *p*H of 6.6. Consequently, 3.3 ml cannot be plated without adversely affecting the *p*H of the medium, upon which the selectivity of the PDA is dependent. Plating 1 ml into each of five plates resulted in a clear medium which inhibited bacterial growth and yielded a mold count per gram comparable to that obtained by platng 3.3 ml in each of three plates. The samples of cottage cheese examined were negative for yeast.

TABLE 1. *Effect of volume of a cottage cheese citrate slurry on microbial growth in acidified Potato Dextrose Agar (PDA)*

Vol of cheese slurry[a]	Vol of PDA at *p*H 3.5 (ml)	PDA + cheese		Bacterial growth[b]	Mold per gram[c]
		Appearance	*p*H		
1.0	10	Clear	4.5	−	8
	20	Clear	4.0	−	8
3.3	10	Cloudy	5.6	+	9
	20	Cloudy	4.9	+	9

[a] Cheese was diluted 1:10 with sterile 2% sodium citrate at 40 C.
[b] −, Negative; +, positive.
[c] All plates incubated at 23 ± 2 C for 5 days.

Three bacterial species inoculated into cottage cheese were recovered quantitatively in acidified PDA (Table 2) by the above APHA method (2). None of the bacteria was inhibited by acidified PDA when 3.3 ml of the citrate-cheese slurry was

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TABLE 2. *Effect of volume of a cheese-citrate slurry on the recovery of bacteria in acidified Potato Dextrose Agar*[a]

Bacteria	Avg no. cells[b] inoculated per plate	Avg. no. cells recovered[c]	
		Cheese (1 ml)	Cheese 3.3 ml
E. coli (ATTC 11840)	11	No growth	11
	110	No growth	141
S. faecalis (M.I.T. strain)	7	No growth	16
	91	No growth	71
S. aureus (U. Md. strain)	5	No growth	1
	12	No growth	5

[a] Cottage cheese was diluted 1:10 with sterile 2% sodium citrate at 40 C.
[b] Based on counts obtained in plate count agar after incubation for 96 hr at 36 C.
[c] Counts in 20 ml of Potato Dextrose Agar were determined after incubation at 23 ± C for 5 days.

plated. However, the medium did remain inhibitory to these bacteria when only 1 ml of the cheese-citrate slurry was plated, since the *p*H of PDA only increased to 4.0 or 4.5 depending on the volume of PDA poured (Table 1).

It is apparent that the APHA method (2) for counting low numbers of yeast and mold in cottage cheese should be modified. The method must be one which inhibits bacterial growth but allows yeast and mold to form colonies. The following modification of the APHA method accomplishes this objective.

Prepare a 1:10 dilution of cottage cheese in sterile 2% sodium citrate preheated to 40 C, as stated in the APHA method (2). Instead of distributing 10 ml evenly among three plates, transfer 1 ml into each of five petri plates and pour with 10 to 20 ml of PDA acidified to *p*H 3.5. Multiply the total number of colonies on the five plates by 2 to obtain the count per gram of cheese. The accuracy may be increased by plating 1 ml into each of 10 petri plates, in which case the total number of colonies will represent the count per gram of cheese. Except for these changes, the microbiological testing of cottage cheese remains as given by APHA.

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